International Antimony Association

EHS GROUP CALL

14 February 2019

Introduction

- Tour de table
- Approval of the conclusions of the last call
- Overview of status of actions agreed in previous call
- Approval of the Agenda
  - Update of the various status
  - Progress of the in vitro inhalation study
  - Progress on the genotoxicity assessment
  - i2a participation in the PSLT workshop
  - Progress tracking of the projects
  - Next events and meetings

Update of the various EHS items

EHS Detailed progress

Please open the xls sheet attached in the invitation – First Tab

Main regulatory driver	Work item
REACH Substance Evaluation	REPROTOX: Bioelution gastric
REACH Substance Evaluation	REPROTOX: Tailored OECD 422 test
REACH Substance Evaluation Classification	LUNG TOX: In vitro lung test (first phase)
REACH Substance Evaluation Classification	LUNG TOX: Genotoxicity research
All	LUNG TOX: Scientific publication on Sb results in ToxTracker
All	LUNG TOX: Scientific publication on Sb genotoxicity

Main regulatory driver	Work item
REACH Substance Evaluation Classification	LUNG TOX: Poorly Soluble Low Toxicity (PSLT) substances debate
REACH Substance Evaluation RMOA	LUNG TOX: Workplace exposure data collection
REACH Substance Evaluation RMOA	LUNG TOX: Exposure data collection in indoor shooting ranges
REACH Substance Evaluation	ADVOCACY: REACH Substance Evaluation
Classification	ADVOCACY: Classification
RMOA	ADVOCACY: RMOA
RoHS	ADVOCACY: RoHS

In vitro inhalation study

Objectives of the reviewed proposal

- Assess lung toxicity potential of the various Sb compounds and the influence of solubility
- Fill knowledge gaps, especially on Sb (V)
- Compare and rank inhalable Sb substances around lung toxicity
- Inform read-across/grouping for carcinogenicity classification
- Avoid a grouped harmonized classification Carc 1B with no specified exposure route following SEv.

Toxicity hypothesis

- Respirable sizes of Sb trigger lung toxicity
- Insoluble or poorly soluble forms of Sb trigger toxicity
- MoA resulting in Sb-induced carcinogenesis is likely to be indirect genotoxicity, instead of direct
  - In rats, this would be initiated by particle overload
  - In mice, this would be initiated through inflammation and reactive oxygen species generation.

Structure of the study

- Development of a an vitro testing divided in 2 phases
  - Phase 1: characterization of the substances with preparation of respirable samples
  - Phase 2; in vitro screening of biological responses to Sb substances.

Phase 1

- Determination of which substance is in a suitable form to be tested and which substance would need to be physically modified to be tested

- Methods to characterize and prepare the substances:
  - Sacanning electron microscopy (morphology and size)
  - Brunauer-Emmer-Teller analysis (surface area)
  - Laser diffusion for PSD (size distribution) and SWeRF (relevant fine particle fraction)
  - Dustiness testing
  - Generation of respirable fraction by grinding

Material

- **9 antimony** compounds will be characterized in their powder form:
  - Antimony metal (Sb)
  - Antimony trioxide (ATO)
  - Antimony trisulfide (ATS)
  - Antimony trichloride (ATC)
  - Antimony trisethylene glycolate (ATEG)
  - Sodium hexahydroxoantimonate (SHHA)
  - Sodium antimonate (SAA)
  - Potassium hexahydroxoantimonate. (PHHA)
  - Antimony pentoxide (APO)
- **Antimony pentachloride** (liquid) cannot be characterized with the methods listed. However, the plan is to include the substance in the phase 2. The way to test is still on discussion due to its properties (potential deposit of liquid vapors in the respiratory tract; corrosivity properties)

Phase 2

- Determination of the toxicity of Sb compounds using an in vitro model, based on the toxicity hypothesis

  (NTP + RAAF document)

- 2 phases
  - Phase 2a: acellular and cellular
    - Toxicity screening on 10 substances, 6 cell phenotypes
  - Phase 2b
    - Selection of substances and cells phenotypes based on the analysis in phase 2a
    - Understanding of the MoA.

Phase 2a – Material and assay

- Determination of the material
  - Physichochemical characterization (research of endotoxine) optimization of the cell culture medium containing the Sb substance)
  - Identification of a suitable particle dispersion protocol
  - Cellular assays will use cell lines from lung respiratory system (alveolar type II epithelial cells and macrophages of human, rat and mouse)
  - Material will be exposed to Sb compounds particulate versus ionic fraction only
- Identification of the oxidative potential
  - Acellular ROS using DCFH
  - Cellular ROS
- identification of a cell death (cytotoxicty and viability)
- Identification of a potential inflammation MoA (Release of proinflammatory mediators

Phase 2a – Benefits and outcomes

- Confirmation of the in vitro observations
- Ranking of the Sb compounds in terms of their oxidative potential
- Informative for read across justification
- Informative for MoA assessment
- Provide BMD for cytotoxicity
- Establish non lethal concentrations for subsequent assay
- Compare effects in rodent versus human
- Contribute to knowledge gaps, including on Sb(V)

Phase 2b (1) on selected substances and cells

- Acellular tests
  - FRAS assay:
    - Determination of the antioxidant responses of human serum in response to Sb compounds, prior to cell interactions.
    - Enable a rudimentary grouping based on based on their active versus passive response
- Cellular test
  - Assessment of oxidative stress (Intracellular GHS and gene expression of HO-1)
    - Quantification of celullar effects generated by the ROS
    - Identification of the intracellular mechanism in response to ROS

Phase 2b (2) on selected substances and cells

- Cellular test
  - MDA quantification
    - Malondialdehyde is a bi-product of lipid peroxidation
    - Determine the oxidative damage of Sb induced ROS on lipids
  - DNA damage COMET
    - Determine the oxidative damage of Sb induced ROS on DNA
  - DNA damage CBMN
    - Determine the DNA damage
    - Determine a potential induction of mutagenicity

Phase 2b – Benefits and outcomes

- Comparison and ranking of antimony substances on their ability in lung tissue to :
  - induce cytotoxicity
  - induce inflammation
  - induce Oxidative stress
- Identification if toxicity is associated with particular physicochemical characteristics.
- Determination if toxicity induced in rodent cells is reproduced in human cells
- Determination if biological responses to Sb are governed mainly by Sb particles or Sb ions
- Establishment of the ROS production and the antioxidant system and if it induces genotoxicity.

Timescale

Tasks	2019				2020				2021			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Phase 1												
Phase 1 reporting and decision												
Phase 2a – assessment of 1 cell phenotypes (3 species)												
Phase 2a – assessment of 2 cell phenotypes (3 species)												
Phase 2a reporting and decision												
Phase 2b assessment*												
Phase 2b reporting												
Project Management												

Figure 11.1 Proposed project timeline for programme of work, deliverables, and key decision points. *The duration of these tests is dependent on which materials and which cell conditions are selected for testing.

Genotoxicity Studies

Significance of in vitro micronucleus induction

- Background: Detection of MN induction in in vitro studies suggesting genotoxic impacts of Sb substances
- Issue : Could it be artificial false positive due to Giemsa staining, not specific to DNA
- Test on ATO and ATC
- Use of acridine orange stain
- Aim: Is MN induction indicative of chromosome damage or staining artefact?

COMET assay results

- Background: positive COMET assay reported by NTP in mouse lung tissue with limited data on cytotoxicity, apoptosis and DNA fragmentation
- Issue: dying cells present a fuzzy halo of fragmented DNA called hedgehogs (recognized as inappropriate to include in results)
- Technical possibility to test using tissue blocks from the NTP Comet assay
- Aim: Assessment of the validity of the comet assay results from ATO mice

Oncogene activation

- Background from the NTP observations, on mice
  - Activated Kras oncogene observed in ATO induced tumors and spontaneous tumors
  - **Activated EGFR oncogene observed only in ATO induced tumors but spontaneous incidence of this oncogene was not determined.**

- hypothesis : possibility of a clonal expansion of preneoplastic cells as a mechanism of tumor formation

- Study of the frequency and progression of oncogene activation in mouse tissue:
  - what is the spontaneous rate of EGFR activation?
  - What is the origin of the EGFR containing tumor?
  - Is clonal expansion of preneoplastic cells a plausible explanation for tumor development?
- Possibility to assess mutagenic change in treated rats, mice and cultured cells and to compare results in vitro and in vivo.

TWINSTRAND™ Biosciences

Poorly Soluble low toxicity particle workshop

Overview

- Background:
  - recent classifications of titanium dioxide and carbon black
  - suggestion to consider PSLTs as a group for safety purposes !!!
- Objective:
  - Provide a forum for experts to discuss and debate the current state of the science on PSLT toxicology.
  - Document where consensus and differences exist among experts. Key topics include:
    - the definition of PSLTs;
    - the particle clearance overload;
    - the design and interpretation of PSLT inhalation studies;
    - the application of existing toxicology and epidemiology studies to PSLT safety.

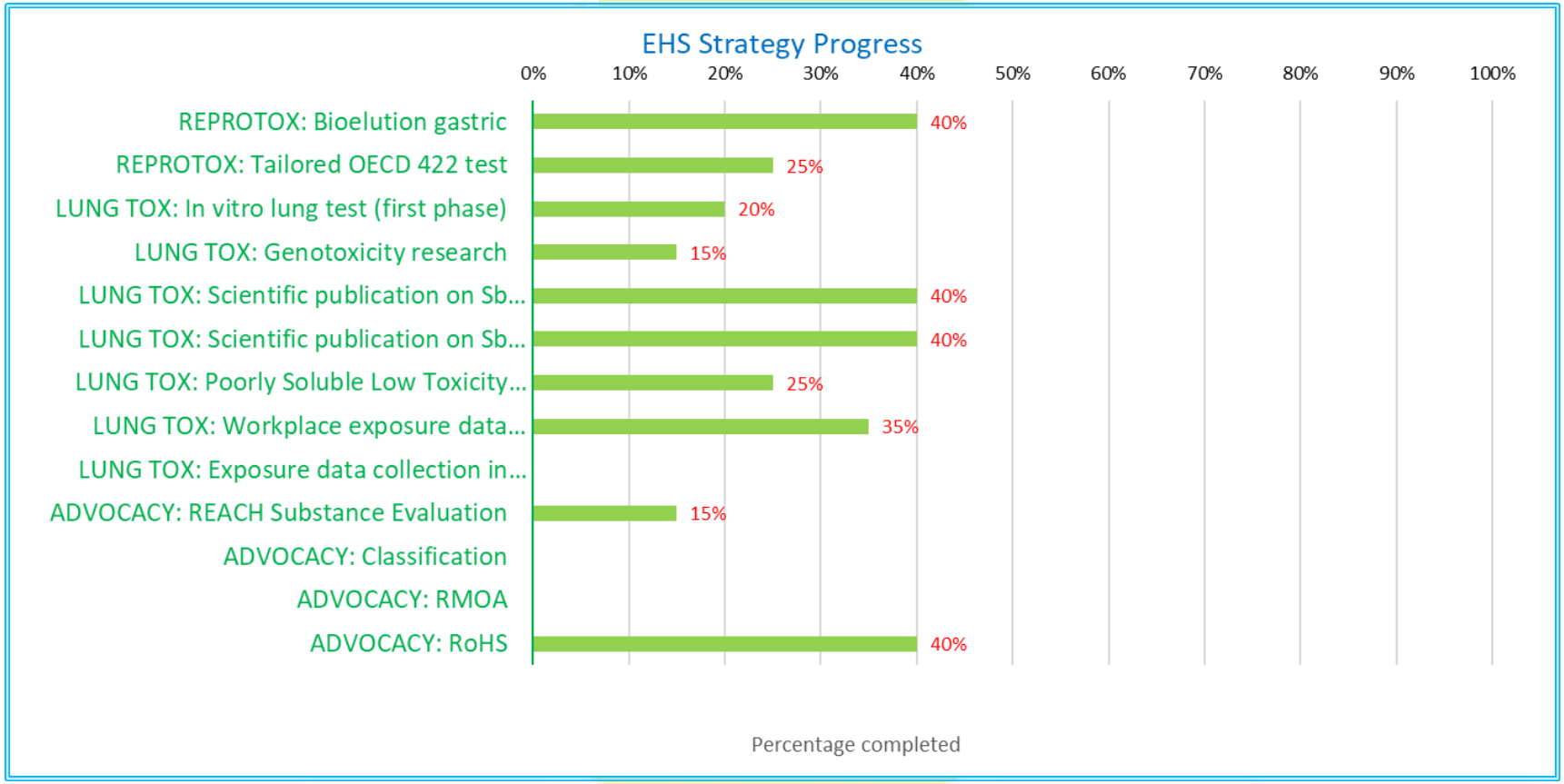
i2a participation

EXPERT WORKSHOP PSLT.

APRIL 1st & 2nd 2019-EDINBURGH (UK)

- 17 experts involved in breakout discussions and preparation of the consensus document
- Observers (including Craig Boreiko for i2a) invited to actively participate in the discussion with sharing points of view
- Raison for i2a to participate:
  - take the chance to be able to influence the discussion
  - ensure that our input is considered.
  - be engaged early and formally in a discussion that will be relevant in the context of the SEv with BAuA,
  - Allow to recognize broadly the distinction between particle effects and chemical effects
  - Allow to discuss the Specific Concentration Limit or SCL assigned to carcinogens

EHS Progress tracking



EHS TIMELME

Please open the xls sheet attached in the invitation – second Tab

Date of status : 31 January 2019	2018				2019											
	Q1	Q2	Q3	Q4	Q1			Q2			Q3			Q4		
					Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Work tasks																
REPROTOX: Bioelution gastric																
REPROTOX: Tailored OECD 422 test					Paused											
LUNG TOX: In vitro lung test (first phase)																
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LUNG TOX: Workplace exposure data collection			BAuA OEL			ACGIH Sb news										
LUNG TOX: Exposure data collection in indoor shooting ranges																
ADVOCACY: REACH Substance Evaluation								SEv Draft Decision	30 days to comment	6-12 mo for Baua to amend the draft decision and to report (amended) decision + 30 d propose amendments + 30 days for registrants to comment proposed am						
ADVOCACY: Classification																
ADVOCACY: RMOA																
ADVOCACY: RoHS	COM Meeting	Stakeholder consultation		Stakeholder consultation												
Calendar of meetings and calls																
i2a BoD						19: Call		25: Call	15: Meeting				30: Meeting			Call
i2a GA						28: Call			16: Meeting				30: Meeting			
i2a Com Group					24: Meeting											
i2a EHS						14: Call		Mail communication		Meeting in Brussels		Call		Call		Call
i2a Toxicologists TF																
i2a Monitoring TF																
i2a RoHS TF																
i2a Public Events						21: Wokplace monitoring workshop								2: Sb Day		
External events							PET Value Chain	- PSLT Workshop - AMI Fire resistance		AsianMeta l?						

Minutes

Minutes

- Attendants
  - Marie-Laure Ledrich (Traxys), Carr Smith (Albemarle), Nathalie Branche (PCL), Stefan Buch (Berzelius), Steven Verberckmoes (Umicore), Sylvaine Duarri D'Haene (Umicore), Oliver Thorsten Schmid (Basf), Francesco Heras (Heras Chemicals), Craig Boreiko, Caroline Braibant (i2a), Marjorie Huppert (i2a).
  - The participants had no comments on last December call. The conclusions were approved.
  - All actions are in progress and in-time
  - The agenda was approved

Minutes

- Bio elution tests in simulated gastric fluids:
  - Samples information are complete (missing information on ATS have been received on the 14/02) .
  - Test will start on 18. 02 at ECTX and analyses are planned to be final by 01.03.
- Reprotox test OECD 422:
  - Test is ready to start after the release of the SEv draft decision, due on April 2019.
  - According to BAuA, the draft decision will include request for similar test.

Minutes

- In vitro lung test:
  - the testing design has been refined following input from the Tox TF and IOM's review.
  - Study foresees two phases
    - 1) Physchem characterization of samples and preparation of respirable samples compatible with in vitro test medium,
    - 2) Assessment of effects of samples in selected cells.
      - Phase 2a) will be a screening of effects
      - phase 2b) will aim to understand the causes and actual mechanisms behind the observed (selected) effects.
  - The preparation for testing can affect the solubility, which may also vary in function of pH. It is important to interpret the results of the in vitro test according to possible influences on the pH and physical preparation on the sample's solubility (and toxicity) will vary during characterization.

Minutes

- In vitro lung test:
  - The method will enable to measure **particle effects** and **ionic effects**. Confirmation of the measurement of Sb release will be explicitly discussed in the next discussion with IOM in order to correctly rank and compare the substances.
  - As APC is a liquid, an alternative approach needs to be considered to be administered to the in vitro model/cells.
  - IOM is implementing final comments into their proposal. The updated protocol will be sent late Feb/early March to the Tox TF for comment/approval and to BAuA before commencement of the tests.
  - The early start of exchanging with IOM and the toxicologists in i2a's Membership enables to be ready to respond to the draft SEv decision (for which i2a will have only 30 calendar days) with a concrete approach in mind.

Minutes

- Genotoxicity research:
  - Various aspects related to the mode of action behind the carcinogenicity observed in animals are worth reviewing or investigating further. Among these:
    - In vitro MN observed in NTP study: are they real MN or staining artifacts?
    - COMET Assay results in NTP study: can they really be interpreted to confirm genotoxic carcinogenicity?
    - Oncogenes activated in NTP study: are these specifically related to the exposure to Sb or can they be spontaneous activations?
  - Proposal from external US partner are under review by C. Boreiko, to be shared with i2a Secretariat by 15 Feb.
- ToxTracker scientific publication:
  - First draft has been received on 11.02 and is currently under review.
  - Next version will be circulated for comment by the Tox TF
- Genotoxicity scientific publication:
  - First draft awaited by end March

Minutes

- PSLT substances workshop
  - TiO_2 and Black Carbon classifications have activated reflection around relevant approach to manage risks related to poorly soluble low toxicity chemicals.
  - C. Boreiko will participate in workshop on behalf of i2a
  - i2a considers to produce a paper comparing the TiO_2 and ATO toxicological profile, exposure, and regulatory cases; in order to better anticipate regulatory developments around ATO on the basis of those for TiO2.

- Workplace exposure data collection:
  - The kick-off event will take place in Brussels on 21st Feb
  - Minimum number of participating companies secured
  - Constant need for more companies, especially downstream users
  - Members should not hesitate to invite customers to join Campaign.

Minutes

- Exposure in indoor shooting ranges:
  - Progressing slowly,
  - Paused until launch of Workplace exposure monitoring campaign.
- REACH Substance Evaluation:
  - Draft decision expected in April, with 30 days to comment.
  - Good working relationship with BAuA.
- RMOA
  - Not yet an i2a a project as such
  - Information being collected with various programs in place + SEA tool will be useful for future performance of RMOA.
- RoHS
  - Meeting with the Commission requested: Industry very concerned about methodologies and overall project run.
  - Initial Dossier for ATO not yet circulated.

Minutes

- ACGIH:
  - New TLV proposal for ATO in circulation: 0.02 mg ***inhalable*** Sb/m^3
  - Intention from i2a to comment by 31 May
  - Justification for proposed TLV not available

- Health and environmental organizations in Canada will start evaluating Sb on the basis of the on-going evaluations elsewhere in the world. i2a identifying contacts to share information with Canadian authorities.

- Timeline of the project is available in the "EHS tracking sheet"

- Calendar of i2a and external relevant events are available in the "EHS tracking sheet."

THANK YOU FOR YOUR PARTICIPATION